Juvenile papillomatosis of the breast (Swiss cheese disease) has frequent associations with PIK3CA and/or AKT1 mutations

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Abstract: Juvenile papillomatosis (JP), the so-called Swiss cheese disease is a rare benign breast disease of young adults. An association (up to 28 %) with breast-cancer within the family of affected patients has been reported. A multinodular cystic breast-mass lesion and calcifications characterizes JP in imaging studies. The histological picture is diverse and comprises multiple intraductal-papillomas, usual ductal hyperplasia (UDH), ductectasias, perifocal sclerosing adenosis and calcification. Patients with complete excision of JP lesions have an excellent follow-up; breast cancer develops only on a very low subset of patients. Molecular background of JP has not been investigated until now. In this study, we addressed mutational analysis of JP cases and correlated these results with follow-up and family-history in context with a comprehensive review of JP literature. We identified 13 cases fulfilling the criteria of JP. All patients were female with a median-age of 38 years (26 to 50 years). Follow-up information was available in 11 of 13 patients. Sufficient paraffin embedded tissue and good DNA quality for next generation sequencing (NGS) was available in 10 patients. Paraffin blocks were microdissected in the area of intraductal proliferative disease, the tissue cores underwent NGS analysis using Oncomine Comprehensive Panel. In 5 of 10 patients, we found PIK3CA mutations, in 2 of 10 patients AKT1 mutations in known hotspot regions. Further mutations in MET, FGFR3, PTEN, ATM, NF1 and GNAS genes were detected in individual patients. Some of these mutations were present at high allelic frequencies suggesting germ line mutations. 2 of 3 patients with positive family history had PIK3CA mutation; one patient with positive family history had an AKT1 mutation. One patient who subsequently developed invasive ductal carcinoma in the contralateral breast possibly had a germ line ATM mutation. Our results confirm hotspot mutations in PIK3CA and AKT1 genes in JP associated with positive family history for breast cancer, although these mutations are not specific for JP. The genetic link between JP, positive family history and subsequent risk of breast cancer, needs further studies.

DOI: https://doi.org/10.1016/j.humpath.2020.02.002

Originally published at:
Guillet, Carole; Rechsteiner, Markus; Bellini, Elisa; Choschzick, Matthias; Moskovszky, Linda; Dedes, Konstantin; Papassotiropoulos, Bärbel; Varga, Zsuzsanna (2020). Juvenile papillomatosis of the breast (Swiss cheese disease) has frequent associations with PIK3CA and/or AKT1 mutations. Human Pathology, 98:64-73.
DOI: https://doi.org/10.1016/j.humpath.2020.02.002

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PII: S0046-8177(20)30020-4

DOI: https://doi.org/10.1016/j.humpath.2020.02.002

Reference: YHUPA 4967

To appear in: *Human Pathology*

Received Date: 27 August 2019

Revised Date: 2 February 2020

Accepted Date: 16 February 2020

Please cite this article as: Guillet C, Rechsteiner M, Bellini E, Choschzick M, Moskovszky L, Dedes K, Papassotiropoulos B, Varga Z, Juvenile papillomatosis of the breast (Swiss cheese disease) has frequent associations with PIK3CA and/or AKT1 mutations, *Human Pathology*, https://doi.org/10.1016/j.humpath.2020.02.002.

Juvenile papillomatosis of the breast (Swiss cheese disease) has frequent associations with PIK3CA and/or AKT1 mutations.

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Running head: PIK3CA and/or AKT1 hot spot mutations in juvenile papillomatosis

Conflict of interest statement: There is no conflict of interest to disclose (neither financial nor non-financial).

Funding: No funding was necessary for this study

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ABSTRACT:

Juvenile papillomatosis (JP), the so-called Swiss cheese disease is a rare benign breast disease of young adults. An association (up to 28 %) with breast-cancer within the family of affected patients has been reported. A multinodular cystic breast-mass lesion and calcifications characterizes JP in imaging studies. The histological picture is diverse and comprises multiple intraductal-papillomas, usual ductal hyperplasia (UDH), ductectasias, perifocal sclerosing adenosis and calcification. Patients with complete excision of JP lesions have an excellent follow-up; breast cancer develops only on a very low subset of patients. Molecular background of JP has not been investigated until now. In this study, we addressed mutational analysis of JP cases and correlated these results with follow-up and family-history in context with a comprehensive review of JP literature.

We identified 13 cases fulfilling the criteria of JP. All patients were female with a median-age of 38 years (26 to 50 years). Follow-up information was available in 11 of 13 patients. Sufficient paraffin embedded tissue and good DNA quality for next generation sequencing (NGS) was available in 10 patients. Paraffin blocks were microdissected in the area of intraductal proliferative disease, the tissue cores underwent NGS analysis using Oncomine Comprehensive Panel.

In 5 of 10 patients, we found *PIK3CA* mutations, in 2 of 10 patients *AKT1* mutations in known hotspot regions. Further mutations in *MET, FGFR3*, *PTEN, ATM, NF1 and GNAS* genes were detected in individual patients. Some of these mutations were present at high allelic frequencies suggesting germ line mutations. 2 of 3 patients with positive family history had *PIK3CA* mutation; one patient with positive family history had an *AKT1* mutation. One patient who subsequently developed invasive ductal carcinoma in the contralateral breast possibly had a germ line *ATM* mutation. Our results confirm hotspot mutations in *PIK3CA* and *AKT1* genes in JP associated with positive family history for breast cancer, although these mutations are not specific for JP. The genetic link between JP, positive family history and subsequent risk of breast cancer, needs further studies.

Key words: Mutations, juvenile papillomatosis, positive family history, breast cancer

INTRODUCTION

Juvenile papillomatosis (JP) of the breast is a rare histopathological entity occurring predominantly in young females (1-3). Usually it presents similarly to fibroadenoma, with localized, multinodular mass on breast examination (4). JP lesions are described to be firmer and larger than fibroadenomas. Tenderness of lesions can occur. In literature, most tumors are described to be located in the upper and lateral quadrant of the breast (5).

Since its first description in 1980, several authors have suggested a correlation between positive family history of breast cancer development and juvenile papillomatosis (2, 5-7). In 26% to 58% of patients with JP, a family history of breast cancer in first- or second-degree relatives has been identified. Positive family history together with recurrent bilateral JP have been identified as risk factor for developing invasive breast cancer (5, 8). Microscopically, epithelial atypia can be found in JP. The grade of epithelial atypia could however not be shown to be associated with consecutive development of breast cancer (5-7).

Until now, there are no data available on molecular alterations in JP and a possible genetic link between JP and consecutive breast cancer development.

Recent studies have explored interactions between *AKT1* and *PIK3CA* signaling pathways in benign and/or proliferative breast lesions and confirmed mutual *AKT1* and *PIK3CA* somatic mutations in non-malignant breast lesions (9-11). The signaling pathway of *AKT1-PIK3CA* plays an important role in normal cell growth, however mutations in this pathway are associated with malignant biological features as invasion and pathological growth in a subset of malignant tumors and in precursor lesions (9-12).

In this study we therefore addressed the question whether JP and its florid epithelial proliferation harbor any hotspot mutations which can be encountered in breast cancer and its precancerous stages. We identified a cohort of 13 JP cases, analyzed clinicopathological characteristics and performed an Oncomine Comprehensive assay on 10 cases.

MATERIALS AND METHODS

Patients' cohort

We identified thirteen consecutive patients that were diagnosed with JP between 1997 and 2018 at the Institute of Pathology and Molecular Pathology, University Hospital Zurich, Switzerland. Patient data were collected after informed consent had been obtained in cases where it was necessary and permission to collect data had been given by Ethical Committee of Canton Zurich (KEK-2012 554). The informed consent was discussed in details with the patients, who gave permission to use the JP samples in a completely anonymous way only for research purposes. As all patients have been treated within certified breast centers, the possibility for genetic counseling at follow-up consultations is regulated by the institutional guidelines.
Patient history, family history and gynecological follow up was obtained either by the hospital pathology data bank or by contacting primary care providers and gynecologists by means of telephone. Patient data was collected in cooperation with Breast Center Zurich Seefeld and the Breast Center at University Hospital of Zurich.

Macroscopic, microscopic and immunohistochemical findings:

All patients underwent surgical excision; old cases underwent surgery without preoperative core- or vacuum biopsies. On gross examination after excision, the breast cut surface showed a prominent cystic appearance embedded in fibrotic and fatty tissue (Fig. 1, upper image). Microscopically, JP encompassess a large spectrum of proliferative findings defined by Rosen et al. in 1980 as: (1) multiple intraductal papillomas with or without epithelial atypia, (2) apocrine oder non-apocrine cysts, (3) extensive intraductal florid hyperplasia, (4) sclerosing adenosis, (5) ductal stasis (1). These histological characteristics are illustrated in low power view (Fig.1 lower image).
All excision specimens in our cohort fullfilled the macroscopic and microscopic criteria of JP.
In cases after 2000, additional immunhistochemistry stains were conducted to prove the florid epithelial proliferations as usual ductal hyperplasia. These stains included basal cytokeratines CK5/6, estrogen receptors (ER) and myoepithelial markes (p63). In all cases the usual hyperplasias showed a mosaic staining pattern with basal cytokeratin, heterogenous staining pattern with ER and preservation of myoepithelial cell around usual ductal hyperplasia and within the papillary proliferations.

Oncomine tumor assay

Sufficient paraffin embedded tissue or DNA of good quality for next generation sequencing (NGS) was available in 10 of 13 patients. Paraffin blocks were punched in the area of proliferative disease and tissue cores underwent NGS analysis using Oncomine™ Comprehensive Assay v3 (OCAv3, Thermo Fisher Scientific). Sample extraction and analysis was performed following manufacturer's instructions. OCAv3 is a pan-cancer targeted NGS panel screening for 161 genes, detecting HotSpot mutations in 87 genes, covering all exons of 48 genes, detecting Copy Number Variations (CNV) in 47 genes and a subset of gene fusions. In this study, only the DNA part of the assay (Mutations and CNVs) was performed.

NGS libraries were sequenced on a S5 (Thermo Fisher Scientific) and the data analyzed using Ion Reporter Software 5.10 with default settings (Thermo Fisher Scientific).

RESULTS

Patient characteristics:

We identified 13 patients diagnosed with JP over the course of 20 years in the University Hospital of Zurich, Institute of Pathology and Molecular Pathology. Information concerning family history and personal history as well as follow-up could be obtained in 11 patients; in two patients, no follow-up data was available. Median age at diagnosis was 38 years, ranging from 26 to 50. Median duration of follow up was 9 years, ranging from 1 to 17 years.

Imaging modalities that lead to excision of tumor sample was breast sonography in 3 patients, breast sonography and mammography in 3 patients, mammography in 1 patient, mammography and magnetic resonance imaging in 4 patients and breast sonography and magnetic resonance imaging (MRI) in one patient.

11 of 13 patients presented with a palpable mass as the leading clinical symptom, in 2 of 13 patients there was a suspicious or unclear mammographic finding.

Histopathologic findings included typical features of JP as described above and defined by Rosen et al (1980) (1). In 3 of 13 patients (23%) flat epithelial atypia was found.

In three patients (Nr. 2,9,10), family history was positive for breast malignancy, in all cases in second grade relatives.

One patient (Nr.5) developed a poorly differentiated invasive ductal breast carcinoma on the contralateral breast four years after excision of a JP tumor. This patient died due to metastatic disease 2 years later. One additional patient had a recurrence of papillomas in the ipsilateral breast. Patient characteristics are summarized in Table 1 and Table 2.

Results next generation sequencing:

In 5 of 10 patients, we found *PIK3CA* mutations and 2 of 10 patients *AKT1* mutations in known hotspot regions. Several further mutations in genes such as *MET, FGFR3*, *PTEN, ATM, NF1 and GNAS* were found in individual patients. The mutations in *MET* and *FGFR3* were detected with a variant allele frequency (VAF) of 47.9% and 50.9%, respectively, suggestive for a germ-line variant. Moreover, these variants were classified in ClinVar with 'conflicting interpretation of pathogenicity' and with 'benign/likely benign'. All other variants were classified as pathogenic/likely pathogenic with two exceptions in the genes *PTEN* and *NF1* where no database entry in COSMIC or ClinVar was found. These two variants are predicted to generate a loss of function on the protein level, since *PTEN* exhibits a frame-shift (c.44_45delGA, p.Arg15fs, patient Nr. 6) and *NF1* a STOP codon (c.4006C>T, p.Gln1336Ter, patient Nr. 5). Interestingly, the *NF1* variant was co-mutated with an *ATM* frame-shift mutation with pathogenic classification (ClinVar ID = 186242, patient Nr. 5). Another loss of function frame-shift variant was detected in patient Nr. 10 in the gene *ATM* (ClinVar ID = 232841). This variant occurred together with a *PIK3CA* mutation (c.3140A>G, p.His1047Arg) with high VAF of 32%.

Two of the three patients with positive family history had *PIK3CA* mutation (patient Nr. 2 and Nr. 10); one patient with positive family history had *AKT1* mutation in a known hotspot region (Nr. 9). The patient (Nr.5) developing contralateral invasive metastatic ductal carcinoma in the follow-up period had an *ATM* mutation in a known hotspot region with a high allele frequency of 48.2%, being suggestive of germ-line mutation rather than being specific for JP. Another *ATM* mutation with a high allele frequency (51%) was detected in a patient with positive family history (Nr. 10) also being suggestive of germ-line mutation. In this patient, there was no evidence of any tumor development within the four years follow-up period. In another patient (Nr. 3) there was a high variant allelic frequency (47.9%) in the *MET* gene (interpreted as conflicting pathogenicity clinical variant), also suggestive of germ line mutation,

however, this patient stayed free of disease after 17 years follow-up period. One further patient (Nr. 12) had *FGFR3* mutations with a high frequency (50.9%, interpreted as benign/likely benign clinical variant) also being suggestive of germ-line mutation, however, this patient also remained free of disease in 9 years follow-up period. In one patient with PIK3CA mutation with high allele-frequency (patient Nr: 2), germline mutation was suspected, although this patient remained healthy in 10 years follow-up.

Relevant NGS results as well as clinical and patient history findings are summarized in Table 2.

DISCUSSION

In this study, we addressed the question whether genetic alterations occur in mammary juvenile papillomatosis and if those mutations could be correlated with findings associated to positive family history.

We could show in our study, that *PIK3CA*, *AKT1* and *ATM* variants together were present in 80% (8/10) of the analyzed JP patients. Several further mutations in genes such as *MET, FGFR3*, *PTEN, ATM, NF1 and GNAS* were found in individual patients. These results support existing literature data on mutational status of normal or proliferative breast tissue, however no data on somatic mutations in JP is available so far.

Juvenile papillomatosis of the breast was probably first described by Kiaer in 1979 and called extreme duct papillomas (13). Rosen reported this lesion as juvenile papillomatosis or Swiss cheese disease in 1980 due to its macroscopic resemblance to cheese from the Swiss Emmental region (1). Rosen defined several histological elements, which are diagnostic criteria for JP. Since its first description, JP has frequently been diagnosed in predominantly young and mostly in female patients, who clinically present with a palpable breast mass (1, 5). However, JP can also occur at higher age (report of 81 years old at first diagnosis) and in men (8, 14-16). A follow-up study by Rosen et al. in 1982 described that JP has a frequent association with positive family history for breast cancer in up to 26% (3). In following studies, a positive family history in patients with JP was reported in 26% to 58% of first- or second-degree relatives. Positive family history together with recurrent bilateral JP have thus been suggested as risk factor for developing invasive breast cancer (1-3, 17) (Table 3). Microscopically, epithelial atypia can be found in JP, however the

grade of epithelial atypia could not be shown to be associated with consecutive development of breast cancer (1-3, 17).

The association between JP and concomitant breast cancer is very low; the first reports from 1979 through 1982 describe a very low occurrence of breast cancer (in the original reported cohorts it was secretory and lobular breast cancer subtype occurring concomitantly) in a very low subset of JP patients (1-3, 13). Since the last two decades several case reports with individual JP cases and only one large study on JP were published (8, 18-27). The number of cases is currently 352 including patients in this study (5, 8, 17, 26, 28-31). 36 reported patients (10.2%) developed breast carcinoma within the available follow-up period and 68 patients (19.3%) had a positive family history for breast cancer (second degree relatives in all cases). (summarized in Table 3).

In our cohort of 13 JP cases, one of the patients developed contralateral invasive ductal breast cancer in the follow up period of 9 years. All other patients are free of breast disease in the available follow-up period. A positive family history for breast cancer (only second degree in all cases) in our series was 23%, which is similar to long existing demographic data of JP.

As to imaging modalities, breast ultrasonography is the preferred method for diagnosis and follow-up of JP patients because of usually the young age at diagnosis (4). On breast ultrasonography, JP usually presents as a hypoechoic mass with small cysts, in a subset of patients there is only an abnormal mammographic finding without corresponding palpable mass (4, 5). Magnetic resonance imaging findings of JP have been described as lobulated as well as well-bordered tubular-shaped masses with cystic and solid components (5). In our cohort, 7 of 13 patients underwent diagnostic ultrasonography prior to diagnostic biopsies / surgery.

Not much is known about the association between JP and hereditary tumor syndromes. It is to assume that *BRCA 1 / 2* mutational status does not apparently play a role in the evolution of JP. However, very limited patients with JP were diagnosed within the context of genetic aberrations in Neurofibromatosis (*NF1)*, Cowden, Noonan or Proteus syndromes (5, 6, 32, 33). In one patient in this cohort, we detected PIK3CA mutation with high allele-frequency suggesting germline mutation, which has been described in patients with Cowden syndrome, Cowden-like

syndrome or in patients with overgrowth disorders in earlier papers (32, 34, 35). Our patient until the age of 45 in 10 years follow-up, showed no clinical findings suggestive of Cowden syndrome or overgrowth disorder.

The genetic background of JP has not been explicitly addressed in any of the previous studies. According to our knowledge, this is the very first study to analyze hotspot and whole gene mutations in the florid epithelial proliferation of JP.

We could show in our study that *PIK3CA* displays a reported hotspot mutation in about 50% of JP cases, accompanied by further hotspot (*AKT1*) or whole gene mutations (*ATM*) in the epithelial anatomical compartments. Several recent papers reported on hotspot mutations in the *PIK3CA* gene in breast lesions, including radial scar, adenomyoepithelioma, columnar cell lesions and further benign proliferative intraductal breast lesions (9, 11, 36-41). Based on the limited recent literature data, *PIK3CA* and *AKT1* mutations occur mutually exclusive in benign breast lesions, probably representing two ends of the same pathway (9-12, 36-41). In our study, this trend of mutually exclusive mutations of *PIK3CA* and *AKT1* has been confirmed.

Further mutations in benign breast lesions affecting the genes *ATM, GNAS and MET* have been reported in the literature and has been also confirmed in our study (40, 42).

In conclusion, the biological relevance of hotspot or whole gene mutations in benign breast lesions is not entirely clear and needs further explorative studies. On one hand, all mutations detected in JP patients in our cohort and in recent literature series in benign proliferative lesion, can occur in a high percentage of malignant and premalignant breast tumors as well (DCIS, invasive breast cancer) (9, 11, 36-41). Therefore, mutations on *PIK3CA* and *AKT1* genes, as detected in our study are not specific for JP. Mutations in high allelic frequencies in individual patients, as detected in *ATM*, *MET*, *FGFR3* genes, which may point to a possible germ-line mutation, would need interpretation in the whole context of the individual patient. As the surrounding uninvolved breast tissue was not included in our analysis, it remains an open question whether the mutations found in this study are either somatic mutations or germline mutations potentially also involving areas of the breast beyond the area with JP. In the absence of comparative data on control tissue not involved by JP, none of the described changes in our cohort can be specifically linked to JP. Mutations in 6 of 13 patients in this study may be germline (due to the high allelic

frequency) and in the remaining 7 patients could be somatic mutations and thus not specific to JP. Based on the data presented in this study, no sufficient genetic association between benign lesions and positive family history for breast cancer in JP patients can be proven at the current time.

ABBREVIATIONS

JP: Juvenile papillomatosis
NGS: Next generation sequencing
DCIS: ductal carcinoma in situ
BC: Breast cancer
ER: Estrogen receptors
NF1: Neurofibromatosis 1

ACKNOWLEDGMENTS

The authors thank the Histology Laboratory of the Institute of Pathology and Molecular Pathology for retrieving all paraffin blocks from the archives.
The study did not require any external funding.
The study was presented as an abstract at the Annual Meeting of the German Pathology Society 2019.

AUTHORS'S CONTRIBUTION

CG (collected clinical information, drafted and finalized the paper), MR (NGS analysis, EB (NGS analysis), MC (provided histological diagnosis), LM (provided histological diagnosis), KD (provided clinical information), BP (provided clinical information), ZV (designed and led the study, drafted and finalized the paper). All authors read and approved the final manuscript.

RERERENCES

1. Rosen PP, Cantrell B, Mullen DL, DePalo A. Juvenile papillomatosis (Swiss cheese disease) of the breast. Am J Surg Pathol 1980; 4, 3-12.
2. Rosen PP, Holmes G, Lesser ML, Kinne DW, Beattie EJ. Juvenile papillomatosis and breast carcinoma. Cancer 1985; 55, 1345-1352.
3. Rosen PP, Lyngholm B, Kinne DW, Beattie EJ, Jr. Juvenile papillomatosis of the breast and family history of breast carcinoma. Cancer 1982; 49, 2591-2595.

4. Ohlinger R, Schwesinger G, Schimming A, Kohler G, Frese H. Juvenile papillomatosis (JP) of the female breast (Swiss Cheese Disease) -- role of breast ultrasonography. Ultraschall Med 2005; 26, 42-45.
5. Cheng E, D'Alfonso T, Patel A, Viswanathan K, Hoda S. Mammary juvenile papillomatosis ("Swiss cheese" disease): Study of 121 cases reiterates need for long-term follow-up. Breast J 2018; 24, 1136-1137.
6. Pacilli M, Sebire NJ, Thambapillai E, Pierro A. Juvenile papillomatosis of the breast in a male infant with Noonan syndrome, cafe au lait spots, and family history of breast carcinoma. Pediatr Blood Cancer 2005; 45, 991-993.
7. Sedloev T, Bassarova A, Angelov K, Vasileva M, Asenov Y. Combination of Juvenile Papillomatosis, Juvenile Fibroadenoma and Intraductal Carcinoma of the Breast in a 15-Year-old Girl. Anticancer Res 2015; 35, 5027-5029.
8. Viswanathan K, McMillen B, Cheng E, D'Alfonso T, Patel A, Hoda SA. Juvenile Papillomatosis (Swiss-Cheese Disease) of Breast in an Adult Male With Sequential Diagnoses of Ipsilateral Intraductal, Invasive, and Widely Metastatic Carcinoma: A Case Report and Review of the Disease in Males. Int J Surg Pathol 2017; 25, 536-542.
9. Kanomata N, Yamaguchi R, Kurebayashi J, Moriya T. Multiplex PCR analysis of apocrine lesions shows frequent PI3K-AKT pathway mutations in both benign and malignant apocrine breast tumors. Med Mol Morphol 2019.
10. Lubin D, Toorens E, Zhang PJ, Jaffer S, Baraban E, Bleiweiss IJ, Nayak A. Adenomyoepitheliomas of the Breast Frequently Harbor Recurrent Hotspot Mutations in PIK3-AKT Pathway-related Genes and a Subset Show Genetic Similarity to Salivary Gland Epithelial-Myoepithelial Carcinoma. Am J Surg Pathol 2019; 43, 1005-1013.
11. Troxell ML, Levine J, Beadling C, Warrick A, Dunlap J, Presnell A, Patterson J, Shukla A, Olson NR, Heinrich MC, Corless CL. High prevalence of PIK3CA/AKT pathway mutations in papillary neoplasms of the breast. Mod Pathol 2010; 23, 27-37.
12. Troxell ML, Brunner AL, Neff T, Warrick A, Beadling C, Montgomery K, Zhu S, Corless CL, West RB. Phosphatidylinositol-3-kinase pathway mutations are common in breast columnar cell lesions. Mod Pathol 2012; 25, 930-937.
13. Kiaer HW, Kiaer WW, Linell F, Jacobsen S. Extreme duct papillomatosis of the juvenile breast. Acta Pathol Microbiol Scand A 1979; 87A, 353-359.
14. El-Saify WM. Florid papillomatosis: A rare cause of bilateral giant breast lumps in the elderly. Breast J 2007; 13, 95-97.
15. Rice HE, Acosta A, Brown RL, Gutierrez C, Alashari M, Mintequi D, Rodriguez A, Chavarrfa O, Azizkhan RG. Juvenile papillomatosis of the breast in male infants: two case reports. Pediatr Surg Int 2000; 16, 104-106.
16. Sanguinetti A, Fioriti L, Brugia M, Roila F, Farabi R, Sidoni A, Avenia N. Juvenile papillomatosis of the breast in young male: a case report. G Chir 2011; 32, 374-375.
17. Lad S, Seely J, Elmaadawi M, Peddle S, Perkins G, Robertson S, Ibach K, Haggar F, Arnaout A. Juvenile papillomatosis: a case report and literature review. Clin Breast Cancer 2014; 14, e103-105.
18. Ferguson TB, Jr., McCarty KS, Jr., Filston HC. Juvenile secretory carcinoma and juvenile papillomatosis: diagnosis and treatment. J Pediatr Surg 1987; 22, 637-639.
19. Hsieh SC, Chen KC, Chu CC, Chou JM. Juvenile papillomatosis of the breast in a 9-year-old girl. Pediatr Surg Int 2001; 17, 206-208.
20. Kafadar MT, Anadolulu Z, Anadolulu AI, Tarini EZ. Juvenile Papillomatosis of the Breast in a Pre-Pubertal Girl: An Uncommon Diagnosis. Eur J Breast Health 2018; 14, 51-53.
21. Munitiz V, Illana J, Sola J, Pinero A, Rios A, Parrilla P. A case of breast cancer associated with juvenile papillomatosis of the male breast. Eur J Surg Oncol 2000; 26, 715-716.
22. Noccioli P, Grossi S, D'Aulerio A, Tavoletta S, Imbriglio G, Cianchetti E. [Juvenile papillomatosis of the breast]. Ann Ital Chir 2003; 74, 13-18; discussion 18-19.
23. Olarinoye-Akorede SA, Farouk B, Liman AA, Kajogbola GA. Giant juvenile papillomatosis of the breast in a Nigerian girl. BMJ Case Rep 2018; 11.
24. Rosen PP, Kimmel M. Juvenile papillomatosis of the breast. A follow-up study of 41 patients having biopsies before 1979. Am J Clin Pathol 1990; 93, 599-603.
25. Sund BS, Topstad TK, Nesland JM. A case of juvenile papillomatosis of the male breast. Cancer 1992; 70, 126-128.
26. Tokunaga M, Wakimoto J, Muramoto Y, Sato E, Toyohira O, Tsuchimochi A, Funasako S, Tachiwada W. Juvenile secretory carcinoma and juvenile papillomatosis. Jpn J Clin Oncol 1985; 15, 457-465.
27. Wang T, Li YQ, Liu H, Fu XL, Tang SC. Bifocal juvenile papillomatosis as a marker of breast cancer: A case report and review of the literature. Oncol Lett 2014; 8, 2587-2590.

28. Bazzocchi F, Santini D, Martinelli G, Piccaluga A, Taffurelli M, Grassigli A, Marrano D. Juvenile papillomatosis (epitheliosis) of the breast. A clinical and pathologic study of 13 cases. Am J Clin Pathol 1986; 86, 745-748.
29. Nonomura A, Kimura A, Mizukami Y, Nakamura S, Ohmura K, Watanabe Y, Tanimoto K, Ikegaki S. Secretory carcinoma of the breast associated with juvenile papillomatosis in a 12-year-old girl. A case report. Acta Cytol 1995; 39, 569-576.
30. Patterson SK, Jorns JM. A case of juvenile papillomatosis, aka "Swiss cheese disease". Breast J 2013; 19, 440-441.
31. Vandeweerd V, Keupers M, Hoste G, Langenaeken T, Neven P. Juvenile Papillomatosis: A Case Report. Eur J Breast Health 2019; 15, 130-134.
32. Ozerdem U, Wells J, Lavi E, Hoda SA. Mammary "Swiss Cheese Disease" in a 26-Year-Old Woman with Cowden Syndrome. Breast J 2016; 22, 115-117.
33. Tan TY, Amor DJ, Chow CW. Juvenile papillomatosis of the breast associated with neurofibromatosis 1. Pediatr Blood Cancer 2007; 49, 363-364.
34. Madsen RR, Vanhaesebroeck B, Semple RK. Cancer-Associated PIK3CA Mutations in Overgrowth Disorders. Trends Mol Med 2018; 24, 856-870.
35. Orloff MS, He X, Peterson C, Chen F, Chen JL, Mester JL, Eng C. Germline PIK3CA and AKT1 mutations in Cowden and Cowden-like syndromes. Am J Hum Genet 2013; 92, 76-80.
36. Ang DC, Warrick AL, Shilling A, Beadling C, Corless CL, Troxell ML. Frequent phosphatidylinositol-3-kinase mutations in proliferative breast lesions. Mod Pathol 2014; 27, 740-750.
37. Delmonico L, Costa M, Fournier MV, Romano SO, Nascimento CMD, Barbosa AS, Moreira ADS, Scherrer LR, Ornellas MHF, Alves G. Mutation profiling in the PIK3CA, TP53, and CDKN2A genes in circulating free DNA and impalpable breast lesions. Ann Diagn Pathol 2019; 39, 30-35.
38. Konstantinova AM, Shelekhova KV, Imyanitov EN, Iyevleva A, Kacerovska D, Michal M, Kazakov DV. Study of Selected BRCA1, BRCA2, and PIK3CA Mutations in Benign and Malignant Lesions of Anogenital Mammary-Like Glands. Am J Dermatopathol 2017; 39, 358-362.
39. Liau JY, Lee YH, Tsai JH, Yuan CT, Chu CY, Hong JB, Sheen YS. Frequent PIK3CA activating mutations in nipple adenomas. Histopathology 2017; 70, 195-202.
40. Volckmar AL, Leichsenring J, Flechtenmacher C, Pfarr N, Siebolts U, Kirchner M, Budczies J, Bockmayr M, Ridinger K, Lorenz K, Herpel E, Noske A, Weichert W, Klauschen F, Schirmacher P, Penzel R, Endris V, Stenzinger A. Tubular, lactating, and ductal adenomas are devoid of MED12 Exon2 mutations, and ductal adenomas show recurrent mutations in GNAS and the PI3K-AKT pathway. Genes Chromosomes Cancer 2017; 56, 11-17.
41. Wilsher MJ, Owens TW, Allcock RJ. Next generation sequencing of the nidus of early (adenosquamous proliferation rich) radial sclerosing lesions of the breast reveals evidence for a neoplastic precursor lesion. J Pathol Clin Res 2017; 3, 115-122.
42. Kato S, Lippman SM, Flaherty KT, Kurzrock R. The Conundrum of Genetic "Drivers" in Benign Conditions. J Natl Cancer Inst 2016; 108.

LEGEND OF FIGURES

Figure 1.

Upper image: Gross appearance of Juvenile Papillomatosis. The breast tissue contains several cysts of different sizes intermingled with fibrotic areas. In some areas cysts are filled with intracystic liquid.

Lower image: Low-power microscopic appearance of Juvenile Papillomatosis. On low-power view, there are several cysts surrounded by sclerosing adenosis and fibrosis. Many cysts contain intracystic mucus and epithelial proliferations and also apparent papillary fronds (Hematoxylin-eosin stain).

Table 1. Clinical features of patients with JP

Clinical characteristics (n=13)	No. of patients total13 (%)
Age (years)	
• Median	38
• Range	26-50
Race	
• White	11
• Asian	2
Menopausal status	
• Premenopausal	13 (100%)
Duration of follow-up (years)	
• Median	9
• Range	1-20
Imaging modality prior to excision	
• Ultrasound	3
• Ultrasound and mammography	3
• Mammography	1
• Mammography and MRI	4
• Ultrasound and MRI	1
Imaging /clinical findings	
• Palpable mass	11 (85%)
• Unclear mammographic lesion	2 (15%)
Localization of JP tumor tissue	
• Left breast	8 (60%)
• Right breast	4 (30%)
• Bilateral	1 (7.6%)
Positive family history for breast cancer	
• First degree relative	0
• Second degree relative	3 (23%)
Abbreviations: JP: juvenile papillomatosis, MRI: magnetic resonance imaging	

Patient number	Age at diagnosis (years)	Initial presentation	Diagnostic workup leading to diagnosis	Family history (FH)	Duration of follow up/ development of breast cancer	Genes	Transcript	Coding	Variant Allele Frequency (%)	Clinical Variant (ClinVar)
1	25	PM	US, TE	unknown	unknown	-	-	-	-	-
2	35	PM, initial presentation at age 7.	US, FNA, TE	Positive FH for breast cancer in aunt (2nd degree relative)	10 years	PIK3CA	NM006218.3	c.3140A>G	39.4	Pathogenic/ Likely pathogenic
3	47	PM, suspect MG	TE	Negative	17 years	MET	NM_001127500.2	c.3029C>T	47.9	Conflicting interpretations of pathogenicity
4	46	PM	MG, MRI, TE	Unknown	Unknown	-	-	-	-	-
5	48	MG	US, FNA, TE	Negative	Contralateral invasive ductal breast carcinoma 4 years after diagnosis of JP; deceased due to metastatic disease 2 years later	ATM NF1	NM_000051.3 NM_001042492.2	c.8395_8404del TTTCAGTGCC c.4006C>T	48.2 5.4	Pathogenic/Likely pathogenic benign/likely benign
6	50	PM	TE	Negative	15 years	PTEN	NM_000314.6	c.44_45delGA	9.7	Pathogenic/Likely pathogenic
7	40	MG	MG, FNA, TE	Negative	11 years	AKT1	NM_001014431.1	c..49G>A	41.4	Pathogenic/Likely pathogenic
8	34	PM	MG, MRI, TE	Negative	5 years	PIK3CA	NM_006218.3	c.1624G>A	25	Pathogenic/Likely pathogenic
9	40	PM; bloody secretion for several months	MG, FNA, TE	Positive, grandmother with mammary carcinoma (age at	5 years	AKT 1	NM_001014431.1	c.49G>A	11	Pathogenic/Likely pathogenic

				diagnosis and histology not known)		GNAS	NM_000516.5	c.601C>T	13.8	Pathogenic/Likely pathogenic
10	39	PM	MG, FNA, TE	Positive (cousin, grandmother)	4 years	PIK3CA ATM	NM_006218.3 NM_000051.3	c.3140A>G c.9001_9002delAG	32 51	Pathogenic/Likely pathogenic Pathogenic
11	23	PM	MG, MRI	Negative	1 year	-	-	-	-	-
12	37	PM	US, FNA, TE	Negative	9 years	PIK3CA FGFR3	NM_006218.3 NM_000142.4	c.3140A>T c.1150T>C	7.6 50.9	Pathogenic/Likely pathogenic Benign/Likely benign
13	39	PM	MG, MRI, TE	Negative	6 years	PIK3CA	NM_006218.3	c.1633G>A	15.6	Pathogenic/Likely pathogenic

Table 2.

Results of next generation sequencing (NGS) including association to clinical characteristics.

Abbreviations: PM: palpable mass, MG: mammographic finding, US: ultrasound, FNA: fine-needle aspiration, TE: total excision,

Table 3.

Original descriptions of juvenile papillomatosis 1979-1985 and summary of published papers since the original disease description in 1985.

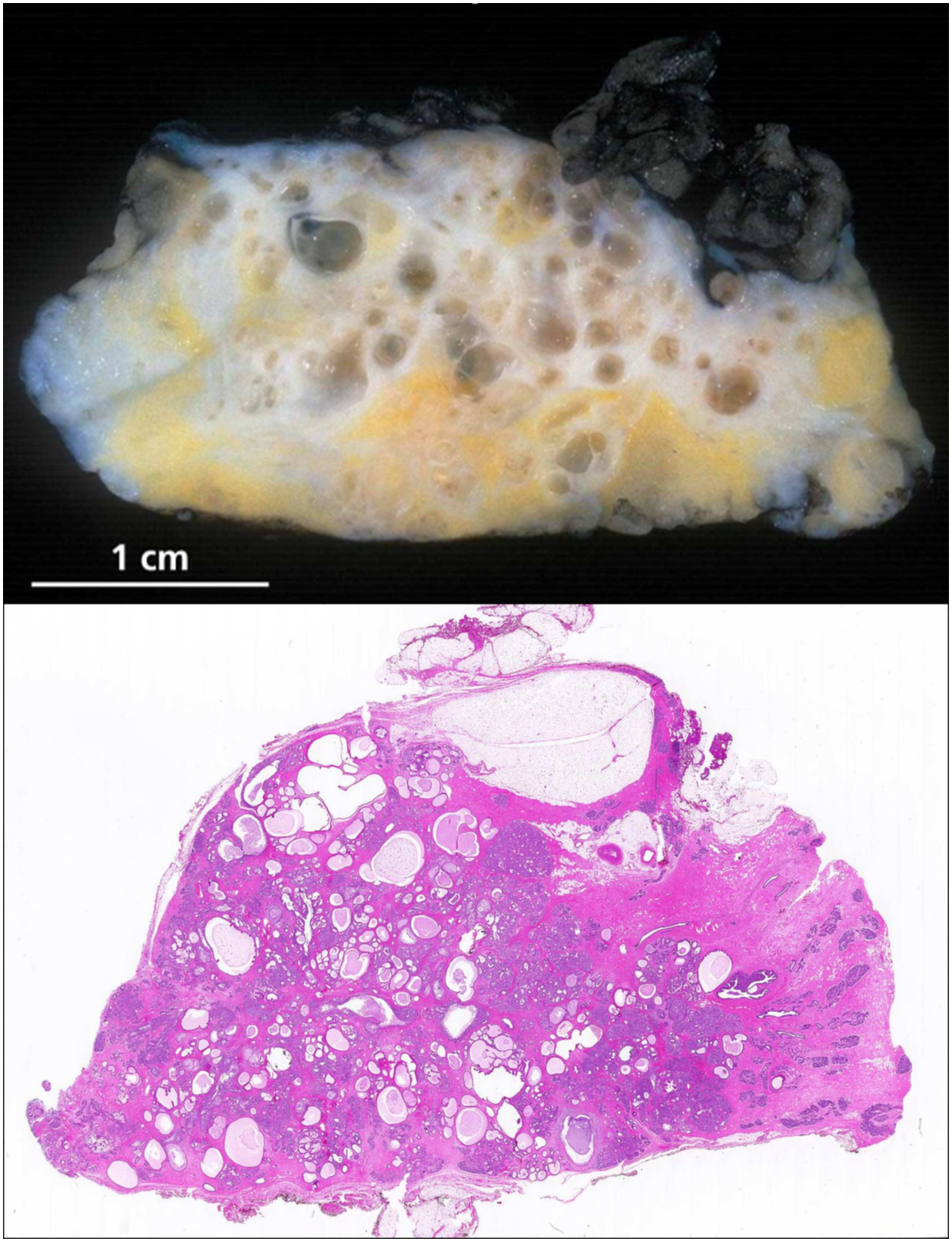
Abbreviations: JP (juvenile papillomatosis), MS (multiple sclerosis), BC (breast cancer), NF1 (neurofibromatosis type 1), NA (not available), FU (follow-up), PH (personal history), FH (family history), PM (palpable mass), LCIS (lobular carcinoma in situ), DCIS (ductal carcinoma in situ), JSC (juvenile secretory carcinoma), IDC (intraductal carcinoma), MDC (microinvasive ductal carcinoma), ILC (invasive lobular carcinoma), IDC (invasive ductal carcinoma).

* The total number of cases in the four papers by Rosen is most likely 180.

Year, Author, Reference number:	N /gender	Age in years (y) or months (mo): mean (range)	Initial presentation (Nr. of cases)	Breast cancer development Nr. of cases (onset in years after initial diagnosis); or synchronous other breast diseases	Family history of breast carcinoma, number or patients (%)	Associated genetic background/ Germline syndrome
1979, Kiaer et al., (ref. 13)	3 / female	14 y (11-17)	PM (3)	2 (27; 11)	NA	
1980, 1982, 1985, 1990, Rosen et al., (ref. 1,2,3,24)*	180 / female	23 y (12-48)	PM (176) Pain (2) Nipple discharge (2)	3 (synchronous LCIS) 2 (associated IDC) 2 (associated JSC) 1 IDC (8) 1 MDC (9)	50 (28%)	
1985, Tokunaga N et al., (ref. 26)	2 / female	13 y, 18 y	PM	2 (associated JSC)	NA	
1986, Bazzocchi et al., (ref. 28)	13 / female	25 y (15 - 42)	PM (13)	2 (associated IDC)	4 (31%)	
1987, Ferguson et al., (ref. 18)	1 / female	6 y	PM	1 (associated JSC)	NA	
1995, Nonomura et al., (ref. 29)	1 / female	12 y	PM	1 (associated JSC)	NA	
2000, Rice et al., (ref. 15)	2 / male	10.5 mo (7-14)	PM (1) Nipple discharge and PM (1)	NA	NA	
2000, Munitiz et al., (ref. 21)	1 / male	33 y	PM	1 (associated IDC)	0	

2001, Hsieh et al., (ref. 19)	1/ female	9 y	PM	0	0	
2003, Noccioli et al (ref. 22)	1 /female	23 y	PM	0	NA	
2005, Ohlinger et al., (ref. 4)	1/ female	16 y	PM	NA	0	
2005, Pacilli et al (ref. 6)	1 /male	1 y	PM	0	1 (100%)	Noonan syndrome
2007, El-Saify, (ref. 14)	1/ female	81 y	PM	NA	NA	
2007, Tan et al (ref. 33)	1 / female	9 mo	PM	NA	NA	Neurofibromatosis 1
2011, Sanguinetti et al., (ref. 16)	1 / male	17 y	PM	NA	0	
2013, Patterson et al., (ref. 30)	1 / female	21 y	PM	NA	0	
2014, Lad et al., (ref. 17)	1/ female	16 y	PM	NA	1 (100%)	
2014, Wang et al., (ref. 27)	1 / female	11 y	PM	0	1 (100%)	
2015, Sedloev et al., (ref. 7)	1/ female	15 y	PM	1 (associated DCIS)	0	
2018, Cheng et al., (ref. 5) (including two published cases: 2017, Viswanathan K et al., (ref. 8), 2016 Ozerdem et al. (ref 32)).	120 / female 1 / male	34.1 y (13-77)	PM: 94 Imaging abnormality: 16	- 1 (associated, ILC) - 4 (associated DCIS) - 1 (associated IDC) - 8 (associated LCIS) - 2 (IDC, 12 and 14 months)	6 (5%)	- 1 case of JP + Cowden disease - 1 case of JP + Proteus syndrome and IDC
2018, Olarinoye-Akorede SA et al., (ref. 23)	1/ female	14 y	PM	NA	0	
2018, Kafadar et al., (ref. 20)	1/ female	11 y	PM	NA	0	
2019, Vandeweerd et al., (ref. 31)	2 / female	22, 23 y	PM	NA	2	
Patients described in this report	13 / female	38 y (26-50)	PM (11) Imaging abnormality (2)	1 (4; IDC)	3 (23%)	

	Total number of cases (female/male)	**Median age (range)**	**Initial presentation (%)**	**Total number of carcinoma development (%):**	**Total number of patients with positive family history of BC (%):**	
	352 (344/6)	26 y (7mo – 81 y)	316 PM (89.7%) 18 Imaging abnormality (5.1%) 2 Pain (0.5%) 2 Nipple discharge (0.5%) 1 Nipple discharge and PM (0.25%)	36/352 (10.2%) 25 associated (7.1%) 11 subsequent development (3.1%); mean: onset after 11 years	68 (19.3 %)	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